

~~x' CGCGAACAGGCTAACCGCACGC, wherein x is nothing or is a sequence recognized by an RNA polymerase and x' is nothing or is a sequence recognized by an RNA polymerase.~~

REMARKS

Claims 24, 35, 39-42, 49, 50, 51, 55 & 56 have been amended to more particularly point out the invention. Claims 24, 35, 41, 51, 55 and 56 have been amended to indicate that one of the first or second oligonucleotide comprises a mixture of two members: one of the members modified at the 3' end to reduce or block extension and the other member either unmodified or differently modified at the 3' end to reduce or block extension. Similarly, claim 50 indicates that the 3' modification reduces or blocks extension by a polymerase. Support for these amendments can be found, for example, at page 24, lines 13-18.

Claims 39-42 have been amended to specify that the claimed kits are for amplifying *Mycobacterium* nucleic acids, and clarify that x is nothing or a sequence recognized by an RNA polymerase. This is accord with Examples 1-6 and 8 of the specification. Claims 49 and 56 have been amended to indicate

that the claimed oligonucleotide comprises a mixture of unmodified members and members modified at their 3' end to reduce or block extension by a polymerase; claim 49 has further been amended to indicate that the mixture may alternatively a mixture of members differently modified at their 3' end to reduce or block extension by an RNA polymerase; these amendments are supported at e.g., page 24, lines 13-18.

New claim 100 is drawn to a kit for amplifying *Mycobacterial* nucleic acid, containing particular oligonucleotide combinations. This claim is supported by, for example, claim 42 as originally filed.

Applicant thanks the Examiner for acknowledging in a telephonic interview conducted on October 18, 1996 and in the Interview Summary mailed October 22, 1996 that the first Office Action did not consider claims 57-99 the Preliminary Amendment filed June 25, 1996, and that examination of these and the present claims will not result in a Final Office Action.

I. The Section 112 Rejections

The Examiner rejected claims 24-42, 48-51, 55 and 56 as allegedly vague and indefinite due to the use of the term "consisting essentially of." The claims have been amended to remove this term, and this rejection is therefore moot.

Claims 24-38, 49, 51 and 56 were rejected as allegedly only enabled for oligonucleotides containing a 3' modification reducing extension by a polymerase. While Applicant does not agree that the teachings of the specification and the well-known art are so limited, in the interest of advancing prosecution of this application the claims have been amended to state that the modification is at the 3' end. Thus, these amendments are also moot.

Accordingly, it is respectfully requested that the rejections under 35 U.S.C. § 112, first and second paragraphs, be withdrawn.

II. The Section 103 Rejections

Claims 24-33, 35, 36, and 54 were rejected as allegedly obvious over Guatelli *et al.*, in view of Schuster *et al.*

Applicant respectfully traverses this rejection.

Guatelli *et al.* describe a three-enzyme isothermal amplification method which, the Examiner agrees, utilizes only unmodified primers. Schuster *et al.* describe an amplification method employing primers modified at their 3' end.

The contested claims are all drawn to compositions and kits containing an oligonucleotide which comprises two promoter-primer members comprising common nucleotide sequences wherein the 3' end of one of the members is blocked to reduce or block extension by a polymerase and the other member is either unmodified or differently modified at its 3' end.

There is nothing in the cited references that suggests conducting an amplification reaction using a mixture of blocked and unblocked promoter primers comprising a common nucleotide sequence. Even further distinguished from the cited references are the compositions and kits of claims 24-33, 35, 36 and 54 made up of mixtures of oligonucleotide members comprising a common

nucleotide sequence and different modifications to the 3' ends. The specification contains ample support for these claim elements; for example, at page 29, (Example 5), and page 28 (Example 28).

Under a proper obviousness analysis, all limitations of the claim must be considered, see e.g., In re Gulack, 217 U.S.P.Q. 401 (Fed. Cir. 1983). Thus, Applicant respectfully submits that when all the limitations of the presently amended claims are considered, this rejection should be withdrawn, since none of the references disclose or suggest, alone or in combination, the limitations of the oligonucleotide mixtures as claimed.

Claims 34, 37 and 38 were rejected as allegedly obvious over Guatelli in view of Schuster and further in view of Hogan et al., U.S. Pat. No. 5,030,557. This rejection is respectfully traversed.

This rejection is predicated on the validity of the Examiner's rejection of the independent claims 24 and 35 over Guatelli in view of Schuster. This rejection has been traversed above and the rejection of claims 34, 37 and 38 must fall as

well. The Hogan '557 patent is a general reference disclosing methods of making helper probes but never mentioning or suggesting the use of helper probes in combination with one or more promoter primer comprising a common nucleotide base sequence, at least one of which contains a 3' modification which lessens or inhibits primer extension by a polymerase. Therefore, the addition of the Hogan '557 patent does not cure the deficiencies of the other two references.

Claims 39-42 were rejected as allegedly unpatentable under 35 USC § 103 over Rogal et al. or Normand et al. This rejection is respectfully traversed.

Rogal discusses PCR amplification of genomic DNA fragments containing a portion of the 16S rRNA gene of various *Mycobacterium* species. The PCR primers reportedly indiscriminately amplify DNA of all of these *Mycobacterial* organisms to yield an amplified DNA containing nucleotide sequence from positions 123 to 273 of the 16S rRNA. Following amplification, the nucleotide sequences of the amplified DNA of different organisms was determined and compared in this region. One such organism is *Mycobacterium tuberculosis*. A figure is

shown comparing the nucleotide sequences of these organisms in this 150 base region.

Rogal does not disclose or suggest the oligonucleotides of claims 39-42. Rogal does not suggest that the 5' end of specific oligonucleotides may be a sequence recognized by an RNA polymerase. Nor does Rogal suggest oligonucleotides of a given size range, as the amended claims now require. The Examiner alleges that the sequence disclosed in Rogal includes SEQ ID NO: 2. However, in order to establish a *prima facie* case of obviousness, the Examiner must show that the reference suggests the particularly claimed oligonucleotides; such suggestion is missing. Thus, Rogal cannot be held to render the claims obvious, and Applicant respectfully requests that the Examiner withdraw this rejection.

Compared to Rogal, Normand presents an even weaker case against claims 39-42 for obviousness. Normand does not discuss *Mycobacteria* at all, but is instead concerned with the organization of the rRNA genes of the actinomycete *Frankia*. The nucleotide sequence disclosed in Normand and referred to by the Examiner is 6481 bases in length. There is nothing in this

reference to suggest making oligonucleotides of about 10 to about 100 nucleotide bases in length having the sequences and optional promoter of the oligonucleotides of claims 39-42. Normand does not suggest nucleic acid amplification or supply any reason for making oligonucleotides as claimed, which comprise SEQ ID NO: 7. Thus, Normand cannot be properly held to render the claims *prima facie* obvious. For this reason the Applicant respectfully requests that the Examiner withdraw this rejection.

Claims 48-51, 55 and 56 were rejected over Rogal or Normand as applied to claims 40-42 above, and further in view of Schuster. The rejection is respectfully traversed. As stated above, neither Rogal or Normand render the independent claims obvious. Also as described above, Schuster does not indicate that the two or more specifically claimed oligonucleotides may comprise common oligonucleotide sequences with one having a 3' modification to reduce or inhibit primer extension and one other either unmodified or having a different 3' modification to reduce or inhibit primer extension. Thus, the addition of Schuster to Rogal or/and Normand does not cure deficiencies of these references.

CONCLUSION

Applicant submits that the rejections to the claims as amended are moot or have been overcome. Accordingly, claims 24-42, 48-51 and 54-100 are in condition for allowance, and a Notice to that effect is respectfully requested. If the fee submitted in connection with this response is incorrect, please charge or credit Deposit Account No. 12-2475 for the appropriate amount.

Respectfully submitted,

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APPENDIX A: PENDING CLAIMS OF APPLICATION 08/480,472

24. A composition comprising:

a nucleic acid comprising a target nucleic acid sequence,

a first oligonucleotide which hybridizes at or near the 3' end of said target nucleic acid sequence,

a second oligonucleotide which hybridizes at or near the 3' end of a nucleic acid sequence perfectly complementary to said target nucleic acid sequence; wherein one of said first and second oligonucleotides comprises a first promoter-primer or a primer, and the other of said first and second oligonucleotides comprises at least two members both comprising a nucleotide sequence in common but different 3' ends, in that the 3' end of one member is modified to reduce or block extension of said oligonucleotide by a polymerase while the 3' end of the other member is either unmodified or differently modified to reduce or block extension of said oligonucleotide by a polymerase,

one or more DNA and/or RNA dependent DNA polymerases, and

an RNA polymerase that recognizes a promoter within one or both of said first or second promoter-primers.

25. The composition of claim 24 wherein said target is RNA.

26. The composition of claim 24 wherein said target is DNA and wherein said first oligonucleotide hybridizes to said target distant from the 3' end of nucleic acid comprising said target.

27. The composition of claim 24 wherein said composition further comprises RNase H activity.

28. The composition of claim 27 wherein said RNase H activity is supplied by an exogenous RNase H from *E. coli*.

29. The composition of claim 24 wherein a reverse transcriptase comprises both said DNA-dependent DNA polymerase and said RNA-dependent DNA polymerase.

30. The composition of claim 29 wherein said reverse transcriptase further comprises said RNase H activity.

31. The composition of claim 24 wherein both of said first and second oligonucleotides comprise promoter-primers, each having a promoter recognized by said RNA polymerase.

32. The composition of claim 24 further comprising one or more of DMSO, dimethylformamide, ethylene glycol, zinc and glycerol.

33. The composition of claim 24 wherein said mixture allows amplification at essentially constant temperature.

34. The composition of claim 24 further comprising one or more helper oligonucleotides.

35. A kit comprising:

a first oligonucleotide which hybridizes at or near the 3' end of said target nucleic acid sequence,

a second oligonucleotide which hybridizes at or near the 3' end of a nucleic acid sequence perfectly complementary to said target nucleic acid sequence; wherein one of said first and second oligonucleotides comprises a first promoter-primer or a primer, and the other of said first and second oligonucleotides comprises at least two members both comprising a nucleotide sequence in common but different 3' ends, in that the 3' end of one member is modified to reduce or block extension of said oligonucleotide by a polymerase while the 3' end of the other member is either unmodified or differently modified to reduce or block extension of said oligonucleotide by a polymerase,

one or more DNA and/or RNA dependent DNA polymerases, and

an RNA polymerase that recognizes a promoter within one or both of said first or second promoter-primers.

36. The kit of claim 35 further comprising an exogenous RNase H.

37. The kit of claim 35 further comprising one or more helper oligonucleotides.

38. The kit of claim 35 further comprising one or more probes able to indicate the presence of said target ribonucleic acid, or its complement.

39. A kit for amplifying *Mycobacterial* nucleic acid, containing at least one of a first and second oligonucleotide; said first oligonucleotide comprising xGCCGTCACCCCACCAACAAGCT, and said second oligonucleotide comprising xGGGATAAGCCTGGGAAACTGGGTCTAATACC, wherein x is nothing or is a sequence recognized by an RNA polymerase and each said oligonucleotide is about 22 to about 100 bases in length.

40. An oligonucleotide of about 20 to about 100 bases in length comprising a nucleic acid sequence selected from the group consisting of xGCCGTCACCCCACCAACAAGCT, xGGGATAAGCCTGGGAAACTGGGTCTAATACC, xCCAGGCCACTTCCGCTAACCC, xCGCGAACAGGCTAACCGCACGC, and their fully complementary sequences of the same length, wherein x is nothing or is a sequence recognized by an RNA polymerase.

41. A kit for amplifying and detecting *Mycobacterial* nucleic acid, containing a first oligonucleotide of about 24 to about 100 bases in length comprising a nucleotide base sequence GTCTTGTGGTGGAAAGCGCTTAG and at least one additional oligonucleotide of about 23 to about 100 bases in length selected from the group consisting of xGCCGGTCACCCCACCAACAAGCT and

x GGATAAGCCTGGGAAACTGGGTCTAATACC, wherein x is nothing or is a sequence recognized by an RNA polymerase.

42. A kit for amplifying and detecting *Mycobacterial* nucleic acid, containing a first oligonucleotide of about 23 to about 100 bases in length comprising a nucleotide base sequence GGAGGATATGTCTCAGCGCTACC and at least one additional oligonucleotide of about 20 to about 100 bases in length selected from the group consisting of x CCAGGCCACTTCCGCTAACCC and x CGCGAACAGGCTAAACCGCACGC, wherein x is nothing or is a sequence recognized by an RNA polymerase.

48. The kit of claim 41 wherein one or more of said sequences has a 3' end modified to reduce or block extension by a polymerase.

49. The kit of claim 41 wherein at least one said oligonucleotide comprises a mixture comprising modified and unmodified members comprising a common nucleotide sequence.

50. The oligonucleotide of claim 40 wherein said oligonucleotide, or said oligonucleotide complementary thereto, has a modification at its 3' end to reduce or block extension by a polymerase.

51. The oligonucleotide of claim 40 comprising a mixture comprising members selected from the group consisting of

- a) 3' unmodified members and members modified at their 3' end to reduce or block extension by a polymerase, and
- b) a mixture of members differently modified at their 3' ends to reduce or block extension by a polymerase.

54. The composition of claim 27 wherein a reverse transcriptase comprises both said DNA-dependent DNA polymerase and said RNA-dependent DNA polymerase.

55. The kit of claim 42 wherein one or more of said sequences has a 3' end modified to reduce or block extension by a polymerase.

56. The kit of claim 42 comprising a mixture comprising unmodified members and members modified at their 3' end to reduce or block extension of said members by a polymerase, wherein said members comprise one or more of said sequences.

57. A nucleic acid hybridization probe, comprising an oligonucleotide from 10 to 100 nucleotides in length which will hybridize with at least 10 contiguous bases of a nucleotide base sequence region of *Mycobacterium tuberculosis* nucleic acid to form a detectable hybridization duplex under selective

hybridization conditions, wherein said region consists of a nucleotide base sequence selected from the group consisting of SEQ ID NOS. 3 and 8, and their fully complementary sequences of the same length.

58. The probe of claim 57, wherein said oligonucleotide is from 15 to 50 nucleotides in length.

59. The probe of claim 57, wherein said oligonucleotide comprises a nucleotide base sequence selected from the group consisting of SEQ ID NOS. 3 and 8, and their fully complementary sequences of the same length.

60. The probe of claim 57, wherein said oligonucleotide consists of a nucleotide base sequence selected from the group consisting of SEQ ID NOS. 3 and 8, and their fully complementary sequences of the same length.

61. The probe of claim 57 containing a detectable label.

62. The probe of claim 61, wherein said detectable label is an acridinium ester.

63. A specifically detectable nucleic acid hybrid formed under selective hybridization conditions between the hybridization probe of claim 57 and a nucleic acid comprising a *Mycobacterium tuberculosis* nucleotide base sequence.

64. A specifically detectable nucleic acid hybrid formed under selective hybridization conditions between the hybridization probe of claim 58 and a nucleic acid comprising a *Mycobacterium tuberculosis* nucleotide base sequence.

65. A specifically detectable nucleic acid hybrid formed under selective hybridization conditions between the hybridization probe of claim 59 and a nucleic acid comprising a *Mycobacterium tuberculosis* nucleotide base sequence.

66. A specifically detectable nucleic acid hybrid formed under selective hybridization conditions between the hybridization probe of claim 60 and a nucleic acid comprising a *Mycobacterium tuberculosis* nucleotide base sequence.

67. An oligonucleotide from 10 to 100 nucleotides in length able to bind to or extend through a region of *Mycobacterium tuberculosis* nucleic acid, wherein said region consists of a nucleotide base sequence selected from the group consisting of SEQ ID NOS. 2, 7, 22 and 23, and their fully complementary sequences of the same length.

68. The oligonucleotide of claim 67 from 15 to 50 nucleotides in length.

69. The oligonucleotide of claim 67, comprising a nucleotide base sequence selected from the group consisting of SEQ ID NOS. 2, 7, 22 and 23, and their fully complementary sequences of the same length.

70. The oligonucleotide of claim 67, consisting of a nucleotide base sequence selected from the group consisting of SEQ ID NOS. 2, 7, 22 and 23, and their fully complementary sequences of the same length.

71. The oligonucleotide of claim 67 which comprises, in the 5' upstream region, an oligonucleotide sequence which is recognizable by an RNA polymerase and enhances initiation or elongation by said RNA polymerase.

72. The oligonucleotide of claim 71, comprising a nucleotide base sequence selected from the group consisting of SEQ ID NOS. 1, 6 and 19.

73. The oligonucleotide of claim 71, consisting of a sequence selected from the group consisting of SEQ ID NOS. 1, 6 and 19.

74. A composition able to amplify *Mycobacterium tuberculosis* nucleic acid, comprising: one or more oligonucleotide from about 10 to about 100 nucleotide bases in length which will, under nucleic acid amplification conditions,

bind to or extend through a region of *Mycobacterium tuberculosis* nucleic acid consisting of a nucleotide base sequence, said region selected from the group consisting of:

- a) SEQ ID NO: 23,
- b) SEQ ID NO: 8,
- c) SEQ ID NO: 7,
- d) SEQ ID NO: 9,
- e) SEQ ID NO: 10, and
- f) the nucleotide sequences perfectly complementary to these sequences.

75. The composition of claim 74 comprising two or more said oligonucleotides.

76. The composition of claim 74 comprising a first oligonucleotide which comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 23, and SEQ ID NO: 7.

77. The composition of claim 76 comprising a second oligonucleotide which comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 23, and SEQ ID NO: 7.

78. The composition of any one of claims 74, 75, or 76, wherein one or more oligonucleotide further comprises, in the 5' upstream region, a nucleotide base sequence which is recognized

by an RNA polymerase and which enhances transcription initiation or polymerization by said RNA polymerase.

79. The composition of any one of claims 74, 76, or 77, further comprising a nucleic acid hybridization assay probe from about 10 to about 100 nucleotide bases in length which will hybridize with at least 10 contiguous bases of a nucleotide base sequence region of *Mycobacterium tuberculosis* nucleic acid to form a detectable duplex under hybridization conditions; said region consisting of SEQ ID NO: 8 or the perfectly complementary sequence thereto.

80. The composition of claim 79, wherein said probe comprises an oligonucleotide with a nucleotide base sequence comprising SEQ ID NO: 8 or the perfectly complementary sequence thereto.

81. The composition of claim 79, wherein said probe comprises an oligonucleotide with a nucleotide base sequence consisting of SEQ ID NO: 8 or the perfectly complementary sequence thereto.

82. The composition of claim 79 wherein said probe contains a detectable label.

83. The composition of claim 82 wherein said detectable label is an acridinium ester.

84. A composition able to amplify *Mycobacterium tuberculosis* nucleic acid, comprising: one or more oligonucleotide from about 10 to about 100 nucleotide bases in length which will, under nucleic acid amplification conditions, bind to or extend through a region of *Mycobacterium tuberculosis* nucleic acid consisting of a nucleotide base sequence, said region selected from the group consisting of:

- a) SEQ ID NO: 22,
- b) SEQ ID NO: 3,
- c) SEQ ID NO: 2,
- d) SEQ ID NO: 4,
- e) SEQ ID NO: 5, and
- f) the nucleotide sequences perfectly complementary to these sequences.

85. The composition of claim 84 comprising two or more said oligonucleotides.

86. The composition of claim 84 comprising a first oligonucleotide which comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 22, and SEQ ID NO: 2.

87. The composition of claim 86 comprising a second oligonucleotide which comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 22, and SEQ ID NO: 2.

88. The composition of any one of claims 84, 85, or 86, wherein one or more oligonucleotide further comprises, in the 5' upstream region, a nucleotide base sequence which is recognized by an RNA polymerase and which enhances transcription initiation or polymerization by said RNA polymerase.

89. The composition of any one of claims 84, 86, or 87, further comprising a nucleic acid hybridization assay probe from about 10 to about 100 nucleotide bases in length which will hybridize with at least 10 contiguous bases of a nucleotide base sequence region of *Mycobacterium tuberculosis* nucleic acid to form a detectable duplex under hybridization conditions; said region consisting of SEQ ID NO: 3 or the perfectly complementary sequence thereto.

90. The composition of claim 89, wherein said probe comprises an oligonucleotide with a nucleotide base sequence comprising SEQ ID NO: 3 or the perfectly complementary sequence thereto.

91. The composition of claim 89, wherein said probe comprises an oligonucleotide with a nucleotide base sequence consisting of SEQ ID NO: 3 or the perfectly complementary sequence thereto.

92. The composition of claim 84 wherein said probe contains a detectable label.

93. The composition of claim 92 wherein said detectable label is an acridinium ester.

94. A helper probe consisting essentially of a nucleotide sequence selected from the group consisting of: SEQ ID NO:9, and SEQ ID NO:10.

95. A helper probe consisting essentially of a nucleotide sequence selected from the group consisting of: SEQ ID NO:4, and SEQ ID NO:5.

96. A probe mix comprising:

a nucleic acid hybridization assay probe comprising an oligonucleotide from 10 to 100 nucleotides in length which will hybridize with at least 10 contiguous bases of a region of *Mycobacterium tuberculosis* nucleic acid to form a detectable hybridization duplex under selective hybridization conditions, wherein said region consists of SEQ ID NO. 8, or its fully complementary sequence of the same length, and

a helper probe.

97. The probe mix of claim 96, wherein said helper probe consists essentially of a nucleic acid sequence selected from the group consisting of: SEQ ID NO:9, and SEQ ID NO:10.

98. A probe mix comprising:

a nucleic acid hybridization assay probe comprising an oligonucleotide from 10 to 100 nucleotides in length which will hybridize with at least 10 contiguous bases of a region of *Mycobacterium tuberculosis* nucleic acid to form a detectable hybridization duplex under selective hybridization conditions, wherein said region consists of SEQ ID NO. 3, or its fully complementary sequence of the same length, and

a helper probe.

99. The probe mix of claim 98, wherein said helper probe consists essentially of a nucleic acid sequence selected from the group consisting of: SEQ ID NO:4, and SEQ ID NO:5.

100. A kit for amplifying *Mycobacterial* nucleic acid, containing a first oligonucleotide comprising
*x*CCAGGCCACTTCCGCTAACCC, and a second oligonucleotide comprising
*x'*CGCGAACAGGCTAACCGCACGC, wherein *x* is nothing or is a sequence recognized by an RNA polymerase and *x'* is nothing or is a sequence recognized by an RNA polymerase.